Acta Protozool. (2004) 43: 281 - 289

ACTA
PROTOZOOLOGICA

# Redescription of *Difflugia tuberspinifera* Hu, Shen, Gu *et* Gong, 1997 (Protozoa: Rhizopoda: Arcellinida: Difflugiidae) from China

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**Summary.** The freshwater testate amoeba *Difflugia tuberspinifera* Hu *et al*. 1997 collected from pond and lake in China, is investigated by light and scanning electron microscopy. This little known taxon is redescribed and its morphology, biometry and ecology are supplied. After carefully comparison with other six similar species including *Difflugia bartoši* Štěpánek, *D. corona* Wallich, *D. corona cashi* Deflandre, *D. corona tuberculata* Vucetich, *D. muriformis* Gauthier-Liévre *et* Thomas and *Netzelia tuberculata* (Wallich) Netzal we believe that the sub-spherical to spherical shell, the mulberry-shaped appearance, the 7-10 apertural tooth-like structures, the short collar and the conical spines numbering from 4 to 8 at the upper equatorial region in *D. tuberspinifera* set it apart from other species. Besides, statistical analysis indicates that *D. tuberspinifera* is a size-monomorphic species characterized by a main-size class and a small size range and the shell height is significant correlated with other morphometric characters at $p < 0.05$ excepting the number of aperture tooth-like structures and the number of spines. Moreover, *D. tuberspinifera* inhabits not only lotic but also lentic environment.

**Key words:** biometry, *Difflugia tuberspinifera*, ecology, morphology, Testacea.

## INTRODUCTION

The testate amoeba genus *Difflugia* established by Leclerc in 1815 is the most extensive one regarding the number of taxa (Cash and Hopkinson 1909, Bartoš 1954, Bovee 1985, Meisterfeld 2000). The taxonomy of this genus is based mainly on differences in shape and size of their shells. As the shell is often opaque, cytoplasmic characters are rarely used. Small differences in shell size, shape, or composition have been sufficient for many authors to describe more than 300 species and about 200 subspecies, varieties, or forms with little regard to the value of the characters used, the previous literature, or the rules of nomenclature. Many of these descriptions are inadequate by modern standards and therefore the determination to species level is extremely difficult, even for the specialist (Meisterfeld 2000). *Difflugia tuberspinifera* Hu, Shen, Gu *et* Gong, 1997 is one of poorly studied species of the genus *Difflugia*.

*Difflugia tuberspinifera* was firstly observed and described in Wujiang River, Guizhou province, China (Hu *et al*. 1997). The empty shells were observed only. According to the original description, "The shell is spherical, with 5 spines at the equatorial region of the body. Aperture: round, petal shape, with 8 dentate lobes. Along

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the margin of the aperture, small sand granules were arranged in a ring regularly. There is a short neck between the aperture and the body of shell. The surface of shell is not smooth, and having many regular blunt protuberances. The sizes of the shell are: diameter of test 115-120 µm, diameter of aperture 57.6 µm, length of spine 38 µm, length of neck 9.6 µm". Unfortunately, no data about the detailed morphometrical characterization and the pseudopodia was presented. During our investigation on the testate amoebae of the Changjiang Valley we have observed an abundant material of living specimens of *D. tuberspinifera* with a high population density. This allows us to make more detailed studies on their morphology, on the variation of shell sizes and on the pseudopodia. The results of our studies are the subject of the present paper.

## MATERIALS AND METHODS

*Difflugia tuberspinifera* was collected from the pond in Xinzhou, Hubei province, China in August 2002 and Mulan Lake (oligotrophic lake, area 105 km$^2$, average depth 18 m, surface water temperature 34°C, and pH 6.0), Hubei province, China in July 2003. Both Xinzhou and Mulan Lake are located in the same climate region (the semitropical humid monsoon climate). The annual mean air temperature of both Xinzhou and Mulan Lake is 16.0°C. The annual mean precipitation is 1250 mm in Xinzhou and is 1100 mm in Mulan Lake. The materials were obtained from the surface water by horizontal hauls of a plankton net made of No. 25 silk bolting cloth (mesh 64 µm in diameter) for about 10 min. Next, they were put in plastic bottles. Examination was immediately made using Leitz optical microscope. Observations on the outline and fine structure of the body were made from living specimens. Some photomicrographs were taken under bright field illumination. After observation of the specimens, they were fixed with Bouin's fluids.

For scanning electron microscopy specimens were first cleaned individually by transference through distilled water using a single-hair brush. Next, they were placed on a cover slip previously cleaned with lint-free tissue. The shells were exposed in air at room temperature until they dried completely. Then the cover slip was mounted on an aluminium-stub using a double-sided adhesive tape and coated with a thin layer gold in Eiko IB-3 Ion Coater before observing. The photographs were obtained from a Scanning Electron Microscopy (X-650 HITACHI, Japan) operating at 20kV.

Nine morphometric characters were measured in our study, namely shell height (character 1 in Fig. 2); shell diameter (character 2 in Fig. 1); aperture diameter (character 3 in Fig. 1); spine length (character 4 in Fig. 2); collar height (character 5 in Fig. 2); rear end length (character 6 in Fig. 2), that is, the distance between the base of conical spine and the shell end; foreside length (character 7 in Fig. 2), that is, the distance between the base of conical spine and the collar; number of aperture tooth-like structures (character 8); number of conical spines (character 9). All measurements were made at middle magnification (320×) using an ocular micrometer. Statistics were performed using the computer program STATISTICA, version 6.0.

## RESULTS

### Morphology

The shell has a sub-spherical to spherical form, composed of fine sand granules, flattish pieces of quartz and muddy particles (Figs 3-14). In apertural view, the shell is circular, furnished with a variable number of conical spines, varying from 4 to 8, usually 5-6. The aperture is terminal, circular, its border denticulated to crenulated with a variable number of small, but perfectly regular tooth-like structures, numbering from 7 to 10, usually 8-9, without any accompaniment of larger quartz grains (Figs 3, 9, 11, 13). In lateral view, the aperture shows a short collar, and the position of the conical spines at the upper equatorial region (Figs 5, 6, 14).

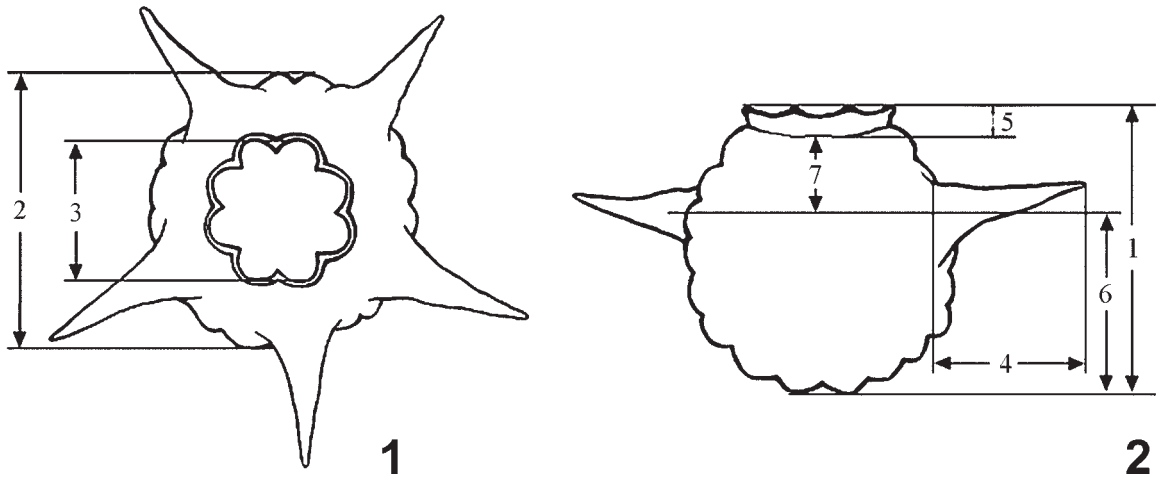
The surface of shell is not smooth and has many regular blunt protuberances. In other words, the shell has a mulberry-shaped appearance (Figs 3-5, 13, 14). However, the shell walls are even in thickness. Accordingly, internal walls of the shell are sunken (Figs 6, 7). The protuberance is composed of small sand granules and flattish pieces of quartz (Fig. 8). No cement structures were recognizable in the scanning electron microscope.

The shell is yellowish to brown, the pseudopodia long, colourless and rather thin, generally 3 to 7 (Figs 10, 14). As the shell is opaque, cytoplasmic characters were not observed.

### Biometry

Table 1 shows the morphometric characterization of *Difflugia tuberspinifera* according to our studies. The values are represented together with those reported in the original description (Table 2). Despite the fact that shell measurements of aperture diameter, spine length, collar height, rear end length and foreside length have high variability (CV between 7.41 and 22.10), shell height and shell diameter are fairly constant and have low variability (CV between 4.47 and 5.36) (Table 1). Numbers of aperture tooth-like structures and conical spines both have low standard error of the mean (0.07-0.11), so does collar height (0.21-0.25) (Table 1).

Size frequency distribution analysis indicates that *D. tuberspinifera* has a main-size class and a small size range. All measured individuals have a shell height




**Figs 1, 2.** Shell outline and position of measured axis used in this study. 1 - shell height; 2 - shell diameter; 3 - aperture diameter; 4 - spine length; 5 - collar height; 6 - rear end length; 7 - foreside length.

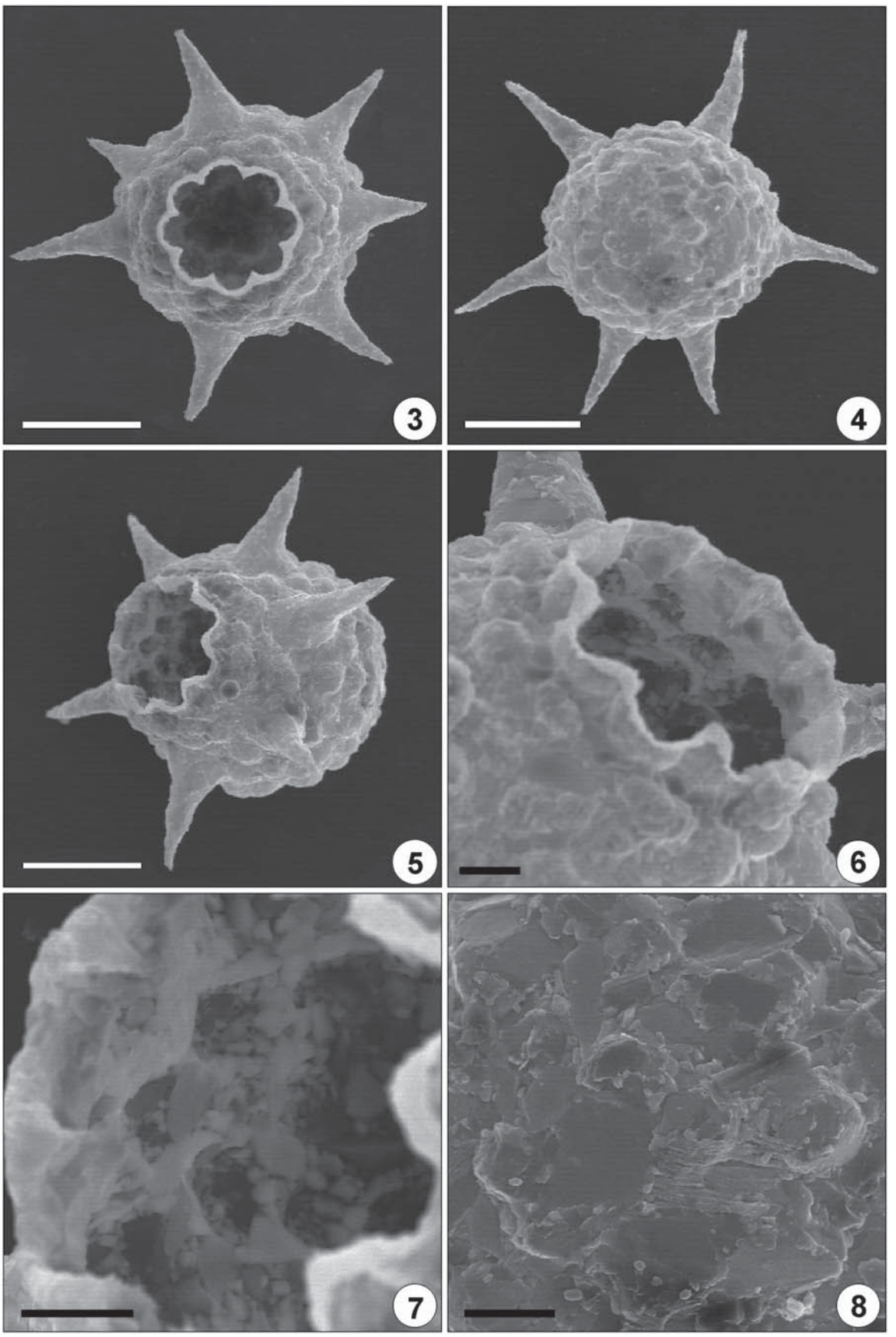
**Table 1.** Morphometric characteristics of *Difflugia tuberspinifera* from pond of Xinzhou (first line for each character) and Mulan Lake (second line for each character).

| Characters[1] | $\bar{X}$ | M | SD | SE | CV | Min | Max | n |
|---|---|---|---|---|---|---|---|---|
| Shell height (1) | 111.4 | 112.0 | 5.71 | 0.81 | 5.13 | 94.0 | 129.0 | 50 |
| | 114.8 | 115.0 | 6.15 | 0.61 | 5.36 | 100.0 | 128.0 | 102 |
| Shell diameter (2) | 109.4 | 110.5 | 5.33 | 0.75 | 4.88 | 94.0 | 118.0 | 50 |
| | 109.5 | 110.0 | 4.90 | 0.49 | 4.47 | 97.0 | 119.0 | 102 |
| Aperture diameter (3) | 49.8 | 49.0 | 5.02 | 0.71 | 10.09 | 39.0 | 64.0 | 50 |
| | 53.0 | 53.0 | 3.93 | 0.39 | 7.41 | 45.0 | 63.0 | 102 |
| Spine length (4) | 28.7 | 28.0 | 6.35 | 0.90 | 22.10 | 15.0 | 42.0 | 50 |
| | 48.2 | 47.0 | 8.79 | 0.87 | 18.24 | 23.0 | 77.0 | 102 |
| Collar height (5) | 10.6 | 11.0 | 1.74 | 0.25 | 16.31 | 8.0 | 15.0 | 50 |
| | 11.6 | 11.5 | 2.15 | 0.21 | 18.51 | 6.0 | 18.0 | 102 |
| Rear end length (6) | - | - | - | - | - | - | - | - |
| | 71.1 | 72 | 6.38 | 0.63 | 8.98 | 53.0 | 86.0 | 102 |
| Foreside length (7) | - | - | - | - | - | - | - | - |
| | 32.0 | 33.0 | 6.79 | 0.67 | 21.20 | 18.0 | 57.0 | 102 |
| Number of aperture tooth-like structures (8) | 8.4 | 8.0 | 0.78 | 0.11 | 9.32 | 7 | 10 | 50 |
| | 8.5 | 8.0 | 0.74 | 0.07 | 8.72 | 7 | 10 | 102 |
| Number of conical spines (9) | 5.6 | 6.0 | 0.61 | 0.09 | 10.82 | 4 | 7 | 50 |
| | 5.7 | 6.0 | 0.68 | 0.07 | 12.00 | 4 | 8 | 102 |

[1]Numbers 1-9 in parenthesis designate features as shown in Figs1 and 2. Data based on randomly selected and character 4 is from only a spine length randomly selected in each shell. Measurements in µm. CV - coefficient of variation in %; M - median; Max - maximum; Min - minimum; n - number of individuals investigated; SD - standard deviation; SE- standard error of mean; $\bar{X}$ - arithmetic mean.

94-129 µm and more than half of them (57%) are within the limits of 111-120 µm. The frequency analysis of the other morphometric characterization (shell diameter, aperture diameter, spine length, collar height, rear end length and foreside length) shows almost the same results. The number of aperture tooth-like structures varies from 7 to 10. In 84% of the measured shells, this number is restricted to 8-9. All measured individuals have conical spines numbering between 4 and 8, but 53% of them are within the limits of 6 and 91% within the ranges of 5-6.

The information in Table 3 illustrates that SH (shell height) is well positively correlated with SD (shell diameter), AD (aperture diameter), SL (spine length),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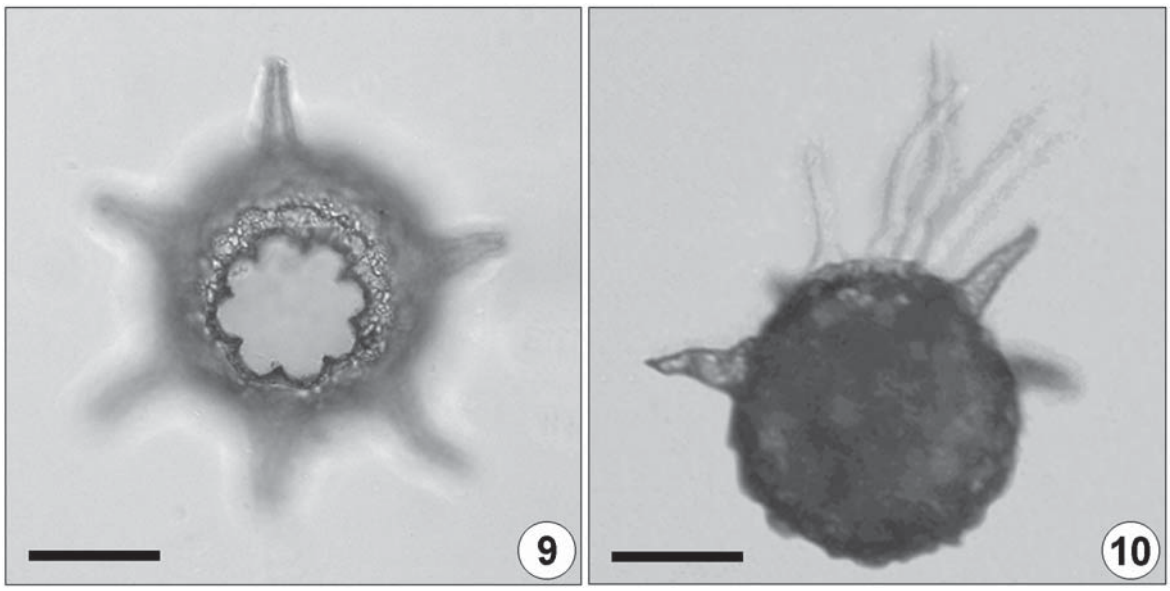



**Figs 9, 10.** LM photographs of *Difflugia tuberspinifera*. **9 -** apertural view, showing shell, aperture and spine shape; **10 -** lateral view, showing pseudopodia. Scale bars 50 µm.

CH (collar height), RL (rear end length) and FL (foreside length), they are significant correlation at $p < 0.001$, $p < 0.05$, $p < 0.001$, $p < 0.001$, $p < 0.001$ and $p < 0.001$ respectively. The AD (aperture diameter) is positive correlation with SL (spine length) at $p < 0.01$ (Table 3). The given Table 3 also shows that RL (rear end length) is positively correlated with SD (shell diameter) and CH (collar height) at $p < 0.001$ and $p < 0.05$ respectively, but highly negatively correlated with FL (foreside length) at $p < 0.001$.

## DISCUSSION

### Morphology, biometry and ecology

The ideal individual of *Difflugia tuberspinifera* from China is constructed from median values of all characters in Figs 13 and 14. In terms of its general appearance, especially the shape and size of shell with a denticular collar and tooth-like structures, conical spines and blunt protuberances, the pond of Xinzhou and Mulan Lake populations both largely correspond with the original description (Figs 11, 12). However, in the original description, only a few empty shells (no observation of pseudopodia) with 8 aperture tooth-like structures and 5 spines were investigated. Furthermore, the variation of the aperture tooth-like structures and the number of conical spines was not mentioned at all (Hu *et al*. 1997). By contrast, in both populations of the pond of Xinzhou and Mulan Lake, there are variable numbers of aperture tooth-like structures going from 7 to 10, and of the conical spines varying from 4 to 8 (Table 1). At the same time, more detailed characters are supplied: the shell is yellowish to brown, opaque; the pseudopodia colourless, long and rather thin, generally 3 to 7.

The variability of shell size in some testate amoeba is high and the biggest individuals can be as twice as large as the smallest in the same taxon (Foissner and Korganova 1995). All morphometric characters in the population from Mulan Lake are a little larger than those in the population from the pond of Xinzhou, especially regarding the aperture diameter and spine length (Tables 1, 2). However, shell size of *D. tuberspinifera* is relatively constant. The regularity of shell height in *D. tuberspinifera* is such that over 93% of all measured individuals (n=152) fall within a range of ±10% of the average value (114 µm). In addition, the shell height is

**Figs 3-8.** SEM photographs of *Difflugia tuberspinifera*. **3 -** apertural view, showing shell, aperture and spine shape; **4 -** bottom view, showing shell, spine and protuberances shape; **5 -** lateral view, showing shell, collar and spine shape; **6 -** showing aperture, collar and protuberances; **7 -** showing sunken internal walls; **8 -** showing the protuberances. Scale bars 50 µm (3-5); 10 µm (6-8).

**Table 2.** Morphometric[1] comparisons of different populations of *Difflugia tuberspinifera.*

| References | Shell height (1) | Shell diameter (2) | Aperture diameter (3) | Spine length (4) | Collar height (5) | Number of aperture tooth-like structures | Number of conical spines |
|---|---|---|---|---|---|---|---|
| Hu *et al*. 1997 (n=?) | 115-120 | 115-120 | 57.6 | 38 | 9.6 | 8 | 5 |
| Present data, Xinzhou pond (n=50) | 94-129 | 94-118 | 39-64 | 15-42 | 8-15 | 7-10 | 4-7 |
| Present data, Mulan Lake (n=102) | 100-128 | 97-119 | 45-63 | 23-77 | 6-18 | 7-10 | 4-8 |

[1]Numbers 1-5 in parenthesis designate features as shown in Figs 1 and 2. Measurements in µm.

**Table 3.** Correlation coefficient between morphometric characteristics in *Difflugia tuberspinifera*. SH - shell height; SD - shell diameter; AD - aperture diameter; SL - spine length; CH - collar height; RL - rear end length; FL - foreside length; NT - number of aperture tooth-like structures; NS - number of conical spines (see Figs 1 and 2).

| | SH | SD | AD | SL | CH | RL | FL | NT | NS |
|---|---|---|---|---|---|---|---|---|---|
| SH | - | | | | | | | | |
| SD | 0.5312*** | - | | | | | | | |
| AD | 0.1676* | 0.0290 | - | | | | | | |
| SL | 0.3026*** | 0.1245 | 0.2614** | - | | | | | |
| CH | 0.3841*** | 0.1556 | 0.1466 | 0.0971 | - | | | | |
| RL | 0.4189*** | 0.3629*** | 0.0141 | 0.1146 | 0.2190* | - | | | |
| FL | 0.3902*** | 0.1758 | -0.0026 | -0.0562 | -0.1739 | -0.6298*** | - | | |
| NT | 0.0031 | 0.0569 | 0.0544 | 0.0606 | -0.1121 | 0.0460 | -0.1720 | - | |
| NS | 0.0488 | 0.1227 | -0.0697 | 0.0577 | -0.0611 | 0.0525 | -0.0479 | 0.0078 | - |

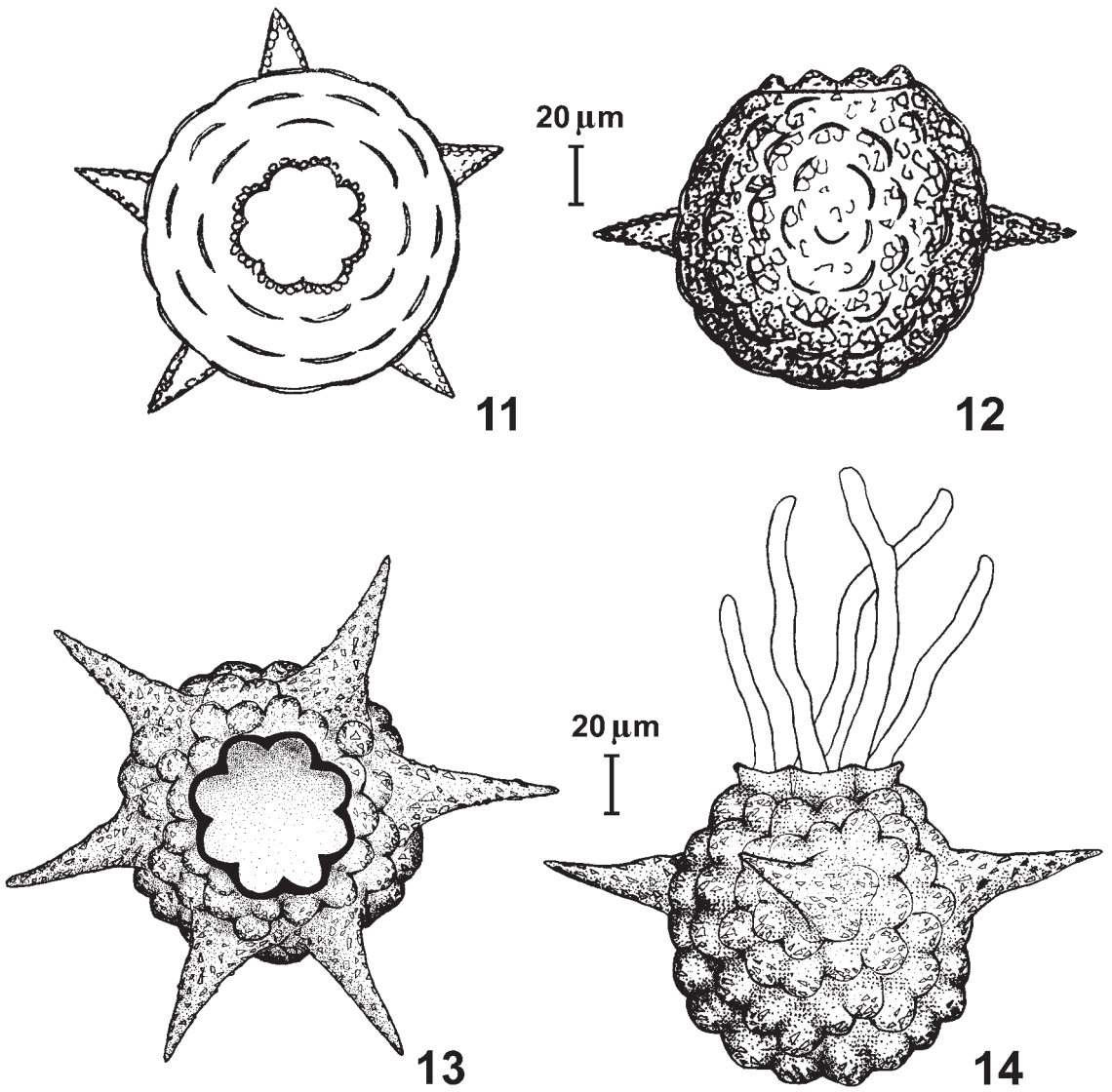
Significant relationship *p < 0.05, **p < 0.01, ***p < 0.001

very important because it is significant correlated with other morphometric characters at $p < 0.05$ with the exception of the number of aperture tooth-like structures and the number of spines (Table 3). According to Hu *et al*. (1997), the shell is furnished with 5 spines at the equatorial region and the number of aperture tooth-like structures is 8. However, in the populations from pond of Xinzhou and Mulan Lake, they both have a variable number of conical spines varying from 4 to 7 (8) and 91% within the ranges of 5-6. Furthermore, the statistical analysis indicates that the spines are not situated in the equatorial region of the shell but in the upper equatorial region. Similarly, the populations from the pond of Xinzhou and the Mulan Lake have a variable number of the aperture tooth-like structures ranging from 7 to 10 with 84% inside the limits of 8-9. Nevertheless, the number of aperture tooth-like structures and the number of conical spines are both quite constant, because they do not follow the change in shell size (for example: shell height), but vary randomly in a limited range (Tables 1, 3). These results have led us to a conclusion that *D. tuberspinifera* is a size-monomorphic species characterized by a main-size class and a small size range.

Since the firstly reported of *D. tuberspinifera* there have been more data about the ecology of this species. Hu *et al*. (1997) pointed out that this species occurred in Wujiang River of Guizhou, China, with water temperature 16°C and pH 6.7. It is evident that it exists in lotic environment. However, our investigation shows that *D. tuberspinifera* inhabits also lentic environments, for example the pond of Xinzhou (neither water temperature nor pH detected) and Mulan Lake (water temperature 34°C and pH 6.0). In these both habitats the quite high

**Table 4.** Comparisons of *Difflugia tuberspinifera* with six similar Testacea species. All measurements in µm. MS - mulberry-shaped; SQ - sand granules and quartz particles; TS - tooth-like structures; ? - data not available; Ab - character absent

| Character | *Difflugia tuberspinifera* | *D. bartoši* | *D. corona* | *D. corona* | *D. corona cashi* | *D. corona tuberculata* | *D. muriformis* | *Netzelia (Difflugia) tuberculata* |
|---|---|---|---|---|---|---|---|---|
| Shell height | 94-129 | 245-385 | 126-190 | 216-225 | 180-230 | 120-160 | 115-150 | 91-150 |
| Shell diameter | 94-118 | 192-297 | 126-177 | 240-260 | 160-180 | 100-140 | 110-138 | 85-132 |
| Aperture diameter | 39-64 | 70 | 51-86 | 160-173 | 80-90 | 50-68 | 31-50 | 30-40 |
| Collar | 6-18 | Ab | present | present | present | Present | 3-7 | >7 |
| Neck | Ab | 35-70 × 75 | Ab | Ab | Ab | Ab | Ab | Ab |
| Shell shape | sub-spherical to spherical | spherical | spherical or ovoid | spherical | sub-spherical | spherical | sub-spherical to spherical | sub-spherical |
| Shell color | yellow to brown and opaque | opaque | brown and opaque | opaque | opaque | ? | yellow to brown and transparent | brown and transparent |
| Shell wall | MS | irregular SQ | smooth SQ | irregular SQ | irregular SQ | MS | MS | MS |
| Aperture | circular | circular | circular | circular | circular | circular | 3-5 lobes | hexagonal |
| Number of lobes | 7-10 | Ab | 10-20 | ? | 12-20 | ? | 3-5 | 6-7 |
| Collar or neck shape | TS | circular | TS | TS | TS | TS | TS | hexagonal with sinuous lobes |
| Number of spines | 4-8 | 4-8 | 2-8 | 4-6 | variable | variable | Ab | Ab |
| Spine position | regularly at the upper equatorial region | regularly around mid-body | randomly at the aboral region | regularly behind mid-body | irregularly behind mid-body | at the aboral region | Ab | Ab |
| Biometry | monomorphic | ? | monomorphic | monomorphic | monomorphic | monomorphic | monomorphic | monomorphic |
| Building material | ? | exogenesis | exogenesis | exogenesis | exogenesis | ? | exogenesis | endogenesis |
| Sample location | China | Slovakia | Yugoslavia and G. Britain | Venezuela | G. Britain and Venezuela | Argentina | Africa | Africa |
| Reference | present study | Bartoš 1954 | Ogden and Hedley 1980, Ogden and Živković 1983 | Deflandre 1926 | Cash and Hopkinson 1909, Deflandre 1926 | Vucetich 1973 | Gauthier-Liévre and Thomas 1958 | Gauthier-Liévre and Thomas 1958, Ogden and Meisterfeld 1989 |




**Figs 11, 12.** Figures of *Difflugia tuberspinifera* copied from the original literature (Hu *et al*. 1997).
**Figs 13, 14.** Ideal individual of *Difflugia tuberspinifera*, constructed from median values of all specimens.

population densities have been observed. Furthermore, *D. tuberspinifera* was a dominant species in many of the investigated samples and its density was by a long way higher than those of other testate amoebae. Probably *D. tuberspinifera* is also a widespread freshwater testate amoeba in the Changjiang Valley, as well as the majority of the known species of the genus *Difflugia*.

**Comparison with similar species**

Considering the morphology, there are some taxa similar to *Difflugia tuberspinifera*. At least six testate amoebae species, namely *D. bartoši* Štěpánek, 1952 (Bartoš 1954); *D. corona* Wallich, 1864 (Deflandre 1926, Ogden and Hedley 1980, Ogden and Živković 1983); *D. corona* var. *cashi* Deflandre, 1926 (Cash and Hopkinson 1909, Deflandre 1926); *D. corona* f. *tuberculata* Vucetich (Vucetich 1973); *D. muriformis* Gauthier-Lievre *et* Thomas, 1958 (Gauthier-Liévre and Thomas 1958) and *Netzelia (Difflugia) tuberculata* (Wallich) Netzel, 1983 (Gauthier-Liévre and Thomas 1958, Netzel 1983, Ogden and Meisterfeld 1989) should be compared with *D. tuberspinifera* (Table 4). None of these six species have the 7-10 apertural tooth-like structures and the conical spines numbering from 4 to 8 at the upper equatorial region. *D. tuberculata* was transferred to *Netzelia* by Netzel (1983) because it

differs from other *Difflugia* species in its ability to endogenously synthesize the building material. *D. bartoši* can be distinguished from other species by its long neck with a round aperture without dentate lobes and conical spines of 1 or 2 cycles regularly around the body. Further, the shell size in *D. bartoši* is much larger than any other six taxa. *D. corona* differs from other species in its 10-20 apertural tooth-like structures and a variable number of conical spines behind mid-body. *D. corona cashi* resembles *D. corona*, but mainly differs in dimension, the sub-spherical shell (shell height > shell diameter) and the irregularly arranged spines. *D. corona tuberculata* is perhaps the most similar organism to the *D. tuberspinifera*. Nevertheless, the spines in *D. corona tuberculata* are situated in the aboral region and they are short in length. In view of the shell wall appearance, *D. muriformis* and *N. tuberculata* are both close to *D. tuberspinifera* in having mulberry-shaped protuberances sometimes masked by the sand-grains of irregular size. However, *D. muriformis* and *N. tuberculata* both lack the spine. Further, aperture has 3-5 lobes in *D. muriformis* and is hexagonal with sinuous lobes in *N. tuberculata*. To summarize: the sub-spherical to spherical shell outline, the mulberry-shaped appearance, the 7-10 apertural tooth-like structures, the short collar, the conical spines numbering from 4 to 8 at the upper equatorial region set *D. tuberspinifera* apart from all other *Difflugia* species.

**Acknowledgements.** We are much grateful to Prof. Meisterfeld R. and Prof. Smith H. G. for their important suggestions and helpful comments. This work was supported by "the Knowledge Innovation Program of the Chinese Academy of Sciences (Grant No. KSCX 2-2-06)", "the National Natural Science Foundation of China (Grant No. 30270185)" and "the Foundation of Donghu Experimental Station of Lake Ecosystems, the Chinese Academy of Sciences (Grant No. 200202)".

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Received on 12th February, 2004; revised version on 19th May, 2004; accepted on 2nd June, 2004